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Sufentanil pre-dosage induces protection against acute focal cerebral ischemia-reperfusion injury in rats

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ABSTRACT

To investigate whether sufentanil pre-dosage induces protection against acute focal cerebral ischemia-reperfusion injury in rats. Method: Forty male SD rats were randomly divided into four groups with 10 rats each. Before establishing animal model, the rats were intraperitoneally injected sufentanil 3 μg/kg (group S1), 6 μg/kg (group S2), 9 μg/kg (group S3) or normal saline (group C). At 30 min after injection, all rats subjected to the right middle cerebral artery occlusion (MCAO) for 120 min, which was followed by reperfusion. The neurological deficit scores (NDS) were evaluated at 24 h after reperfusion. Infarct volume, as a percentage of volume at normal cerebral hemisphere, was determined by TTC staining. Results: At 24 h after reperfusion, the NDS was higher and infarct volume was less in group S1 than those in groups of S2, S3 and C (P < 0.05). There were no significant differences in NDS and infarct volume among groups of S2, S3 and C. Conclusion: Sufentanil preconditioning can protect rat brain against ischemia-reperfusion injury.

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KEYWORDS

Sufentanil;
Cerebral ischemia-reperfusion injury.

INTRODUCTION

The rate of death and disability of cerebral trauma and cerebrovascular misadventure is high^[1]. It has a strong impact on the health and life quality of human beings. Although there are many intervening measures such as hypothermia therapy, glutamate receptor retardant which has the neuroprotective effect etc.^[2], there is a lack of safe and effective drugs and methods to

decrease cerebral ischemic injuries. Sufentanil is a new type μ opiate receptor agonist. It is widely used in clinical anesthesia for its unique and outstanding merits. Researches on animals show that sufentanil can simulate the effect of ischemic preconditioning and induce the generation of myocardial ischemia endurance^[3]. There is no literature report on the cerebral protection effect of sufentanil preconditioning. Therefore, this research is conducted to observe whether sufentanil pre-

conditioning has the cerebral protection effect through rat models of focal cerebral ischemia.

MATERIALS AND METHODS

Experimental animals and grouping

40 male Sprague- Dawley rats (provided by Animal Experiment Center of Medical School of Yangtze University) with weight in 400 ± 20 g. The using of animals is in line with conservation regulations for conservation regulations. These rats are divided into four groups at random: separately inject $3\mu\text{g}/\text{kg}$ (group S1), $6\mu\text{g}/\text{kg}$ (group S2), $9\mu\text{g}/\text{kg}$ (group S3) of sufentanil and normal saline of equivalent amount to the control group (group C) through intraperitoneal injection 30min before ischemia molding. All animals are fasting for 12 hours before operation, but they are free for drinking.

Modeling of focal cerebral ischemia

30min after intraperitoneal injection, form middle cerebral arterial occlusion (MCAO) on right internal carotids of all rats by nylon suture method. After animals are anaesthetized, cut from the middle neck to expose the right arteria carotis communis, external carotid artery and internal carotid; ligate arteria carotis communis and external carotid artery; cut a incision at the lower part of the arteria carotis communis, put a nylon wire which is fired to obtuse by a alcohol burner 17-18mm into the internal carotid until slight resistance is felt. Draw out the nylon wire 120min after occlusion. Perfuse again after recovering.

Observational indexes

Animal recovery and neurological dysfunction assessment (NDS):after animals are recovered from anesthesia, put them back to cages for free eating and drinking. 24hours after reperfusion of cerebral ischemia, observers who are blind to the grouping shall assess and record the neurological dysfunction score (NDS) by referring to the Garcia scoring method, namely the 6-grade scoring method: grade 1, spontaneous activity (0-3 scores); grade 2, symmetrical limb activity (0-3 scores); grade 3, fore limb stretching situation (0-3 scores); grade 4, climbing situation (1-3 scores); grade 5, noumenal body reaction (1-3 scores); grade 6, whis-

ker reaction (1-3 scores). Score for each rate is the total of the above scores. Rat with 3 scores to the lowest and 18 scores to the highest is normal.

TTC staining and cerebral infarction focus measurement

The NDS is completed 24 hours after reperfusion. Cut heads of deeply anaesthetized animals. Take out brains of rats rapidly. Put them in the brine ice for 10min. Cut the coronal plane into thick brain slices of 2mm, put them into 2% TTC solution rapidly for 30min of staining, then fix them with 10% formalin solution. Take photos of these brain slices with a digital camera (Sony, T700) and input these photos into the computer. Then use the image processing software (Adobe Photoshop cs 8.0) to compute the volume percent of cerebral infarction (the pink area is normal brain tissue and the white area is infarct), namely the percent of infarct focus in the opposite normal cerebral hemisphere.

Statistic analysis

Data are analyzed by the SPSS 13.1 statistical analysis software. Measurement data are presented by mean+ standard deviation ($\bar{x} \pm s$). One-way analysis of variance is adopted for the cerebral infarction volume percent. NDS is represented by median (scope) and tested by Mann-Whitney U.

RESULTS

NDS scoring 24 hours after reperfusion of four groups of rats, the NDS score of group S1 is apparently higher than that of group S2, S3 and C ($P < 0.05$). There is no statistical significance in differences among group S2, S3 and C (TABLE 1).

TABLE 1 : NDS 24 hours after reperfusion of four groups of rats

Groups	number of elements	NDS (score)
S1	10	12(9~ 14)
S2	10	10(8~ 12)*
S3	10	9(7~ 10)*
C	10	9(7~ 11)*

Compared with group S1, * $P < 0.05$

Cerebral infarction volume percent

24 hours after reperfusion of four groups of rats,

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the cerebral infarction volume percent of group S1 is apparently lower than that of group S2, S3 and C ($P < 0.05$). There is no statistical significance in differences among group S2, S3 and C (TABLE 2).

TABLE 2 : Cerebral infarction volume percent 24 hours after reperfusion of four groups of rats (% , $\bar{x} \pm s$)

Groups	Number of elements	Cerebral infarction volume percent
S1	10	32.7±5.30
S2	10	45.90±4.69 [△]
S3	10	43.90±1.47 [△]
C	10	52.50±2.41 [△]

Compared with group S1, $\Delta P < 0.05$

DISCUSSION

3 μ g/kg, 6 μ g/kg and 9 μ g/kg sufentanil pre-administration is selected to observe its influence on the transient ischemic attack on rat, the results indicate that 3Lg.lk pre-administration can reduce the volume of cerebral infarction of rat caused by MCAO.

Opioid agonist is a common drug used in anesthesia and analgesia. The previous researches have shown that morphine can generate preconditioning effect and reduce the ischemic injury of myocardium and brain^[4-6].

For a long half-life period of morphine, it is still unclear whether this protection role is from its direct action or the preconditioning protection effect triggered by it^[5]. There have been previous researches showing that the preconditioning of both fentanyl and sufentanil has a role of myocardial protection. Currently, the various known opioid receptors include μ , δ , κ and ORL1. δ opioid receptor plays a key role in neuroprotection. The mechanism of both hypoxic preconditioning^[9] and low frequency electro-acupuncture preconditioning^[10] cerebral protection role is related with δ opioid receptor. In recent years, the role of κ opioid receptor in preconditioning has also been attached attention to. In rats and marmosets, κ agonist Enadolol (C1977) can reduce the glutamic acid released by neuron under 4-aminopyridine excitement and relieve the cerebral injury mediated by excitatory amino acid^[11].

Sufentanil is a specific μ opioid receptor agonist.

There has been research^[12] finding that the preconditioning of another μ opioid receptor agonist fentanyl also has a protection role to the focal cerebral ischemia-reperfusion injury of rates. The specific mechanism on the previous administration of fentanyl to cerebral ischemia reperfusion injury is still unclear. Garca Fuster et al.^[13] found that opioid drugs could inhibit Fas-related death domain protein, so as to promote anti-apoptosis. The regulation of anti-apoptosis approach might be one of the mechanisms for previous administration protection role of sufentani. As the organ protection induced by sufentani preconditioning has universality (heart and brain etc.), sufentani has a significant application prospect clinically. For example, when cardiovascular and cerebrovascular disease patients need surgical operation, sufentani can be selected as the narcotic. In addition to the function of anesthesia and analgesia, sufentani can also relieve the positional cerebral and myocardial ischemic injuries. In a word, the experiment demonstrates that 3 μ g/kg sufentani can also improve the neurological deficits of rats and the infarct size is small, having a protection significance to cerebral ischemia-reperfusion injury.

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